Supplementary Information 1

Epithelial cell subsets, particularly enteroendocrine cells, are directly exposed to microbial signals and are capable of sensing and transmitting luminal input to EANs via synaptic transmission[1] and/or via secretion of neuropeptides and neurotransmitters[2]. Recent evidence pointed to a crucial role for the microbiota in modulating three major pathways: upregulation of serotonin[3], downregulation of L cell–derived glucagon-like peptide 1 (GLP-1)[4], and up or downregulation of peptide YY (PYY)[5,6]. Because secretion of these molecules is particularly enriched in the distal small intestine and colon, locations also enriched for SCFA production, we examined their role in the microbial modulation of sympathetic neurons. We addressed a possible suppressive role for epithelial–derived serotonin by crossing *Tph1*$^{fl/fl}$ mice with mice expressing inducible Cre^{ER} under the villin promoter (Villin^{Tph1}). Conditional depletion of the key enzyme for serotonin production in gut epithelial cells resulted in a 43-52% reduction in total colonic serotonin upon tamoxifen administration, consistent with previous literature and in line with GF and antibiotic–treated levels[7,8] (Extended Data Fig. 4b, c). Nevertheless, a 50% reduction in gut-derived serotonin did not result in changes in intestinal motility or cFos+ neurons in the CG-SMG (Extended Data Fig. 4d, e). In contrast, administration of the GLP-1R agonist Exendin-4 increased cFos+ neurons in the CG-SMG, as well as the total gastrointestinal transit time (Extended Data Fig. 4f-h). The effect of Exendin-4 on motility was likely mediated by sympathetic activation, as catecholamine blockade normalized gastrointestinal transit time (Extended Data Fig. 4i). To assess whether this pathway was required for microbial regulation of gut sympathetic neurons, we first treated mice with Exendin (9-39), a GLP-1R antagonist, after administration of streptomycin; we observed unaltered CG-SMG cFos levels (Extended Data Fig. 4j). To further evaluate whether GLP-1R signalling is required for microbial depletion-associated sympathetic activation, we treated *Glp1r*$^{-/-}$ mice with broad-spectrum antibiotics or Splenda. We observed similar numbers of cFos+ neurons in the CG-SMG isolated from the antibiotic-treated group as compared to wild-type mice, overall indicating that GLP-1 is sufficient to drive sympathetic activity, albeit not required for microbial–dependent modulation of gut sympathetic neurons (Extended Data Fig. 4k). Finally, PYY administration did not activate the CG-SMG in SPF animals, but efficiently prevented the increase in cFos+ CG-SMG neurons following treatment with streptomycin (Extended Data Fig. 4l, m). These data suggest that the neuropeptides GLP-1 and PYY can modulate activity of gut sympathetic neurons and, in the case of PYY, may contribute to microbial regulation of gut sympathetic activity.

References

1 Kaelberer, M. M. *et al.* A gut-brain neural circuit for nutrient sensory transduction. *Science* **361**, doi:10.1126/science.aat5236 (2018).

2 Gribble, F. M. & Reimann, F. Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium. *Annual review of physiology* **78**, 277-299, doi:10.1146/annurev-physiol-021115-105439 (2016).
3 Hsiao, E. Y. *et al.* Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* **155**, 1451-1463, doi:10.1016/j.cell.2013.11.024 (2013).
4 Wichmann, A. *et al.* Microbial modulation of energy availability in the colon regulates intestinal transit. *Cell host & microbe* **14**, 582-590, doi:10.1016/j.chom.2013.09.012 (2013).
5 Browning, K. N. & Lees, G. M. Inhibitory effects of NPY on ganglionic transmission in myenteric neurones of the guinea-pig descending colon. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* **12**, 33-41 (2000).
6 Newman, K. D., Mhalhal, T. R., Washington, M. C., Heath, J. C. & Sayegh, A. I. Peptide Tyrosine Tyrosine 3-36 Reduces Meal Size and Activates the Enteric Neurons in Male Sprague-Dawley Rats. *Dig Dis Sci* **62**, 3350-3358, doi:10.1007/s10620-017-4788-3 (2017).
7 Kim, H. *et al.* Generation of a highly efficient and tissue-specific tryptophan hydroxylase 1 knockout mouse model. *Scientific reports* **8**, 17642, doi:10.1038/s41598-018-36097-6 (2018).
8 Yano, J. M. *et al.* Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264-276, doi:10.1016/j.cell.2015.02.047 (2015).